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The pattern recognition molecule collectin-L1 in critically ill children

Running title: Collectin-L1 in critically ill children

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Abstract

Background

Critically ill children are prone to nosocomial infections, which may lead to adverse outcome. Low serum concentrations upon admission to the pediatric intensive care unit (PICU) of the MASP-3 protein of the lectin pathway of complement activation have been associated with risk of infection and prolonged need for intensive care. We hypothesized that also a low upon-admission concentration of collectin-L1 (CL-L1), a novel member of this pathway, is independently associated with these adverse outcomes.

Methods

We quantified the serum concentrations of CL-L1 in 81 healthy children and in 700 critically ill children upon PICU admission.

Results

CL-L1 concentrations were significantly lower in the critically ill children as compared with the healthy children. However, corrected for baseline characteristics, risk factors and several lectin pathway proteins, a *higher* CL-L1 concentration upon PICU admission was independently associated with an increased risk of acquiring a new infection and with a prolonged time to PICU discharge. In contrast, a low MASP-3 concentration remained independently associated with these adverse outcomes.

Conclusion

A high serum CL-L1 concentration in critically ill children upon PICU admission is associated with an increased risk of infection and prolonged need of intensive care, and counteracts the protective effect of having a high MASP-3 concentration.

Introduction

Critically ill children are particularly vulnerable to potentially life-threatening nosocomial infections. Indeed, secondary infections of diverse origins have been reported to occur in approximately 30% of the patients (1). The innate immune system is an ancient, conserved defense mechanism, which provides the immediate response that is of paramount importance for the protection against invading pathogens as well as for the repair of tissue damage (2). The complement system, which consists of soluble proteins acting as pattern recognition receptors, proenzymes, cell surface complement receptors and regulatory proteins, is an essential part of innate immunity (3). The complement system is important for fighting infections, for clearing immune complexes and cellular waste and for linking innate to adaptive immunity. Insufficient as well as excessive activation of this system can be detrimental and thus, health depends on a subtle balance (4). Defects in components of the inflammatory complement system have been linked to increased susceptibility to infections in young children with associated pathologies (5, 6). Also, depletion of complement factors has been shown to be associated with adverse outcome in abdominal sepsis (7, 8). On the other hand, excessive inflammation, induced by trauma or infection, has also been linked to worsening of organ function and shock (9, 10).

The complement system is redundant and may be activated in different ways. One way is via the so-called lectin pathway, which recognizes patterns of foreign carbohydrate structures (e.g. on pathogens). This pathway may be initiated by different pattern recognition molecules, including mannan-binding lectin (MBL), one of the ficolins (H-, M-, and L-ficolin) and the collectins (11, 12). This pathway also exhibits the duality of the system, as both insufficient and excessive activation may be detrimental. For example, an increased susceptibility to secondary infections or complications was shown with deficiency in MBL or ficolins in children with cancer and in neonates with NEC [6, 13, 14]. In contrast, increased levels of these innate immunity proteins, which could possibly be explained by

genetic polymorphism, may contribute to exaggerated inflammatory responses as seen in the acute respiratory distress syndrome [15, 16]. Overall, data on the lectin pathway of complement activation in critically ill children are scarce.

We previously studied MBL, M- and H-ficolin, and their MBL-associated serine proteases (MASPs) in a large group of critically ill children (17). Serum concentrations of these proteins were lower in critically ill children upon admission to the pediatric intensive care unit (PICU) as compared with age-matched healthy controls, except for higher concentrations of M-ficolin. In particular a low MASP-3 level upon admission was independently associated with the risk of acquiring a new infection and with the risk of needing prolonged intensive care.

Within the last 15 years, a novel group of pattern recognition molecules has been discovered, which includes collectin-L1 (CL-L1, also named collectin-10) in addition to CL-K1 (also named collectin-11) and CL-P1 (12, 18-21). So far, very little is known about the biological function of this class of collectins. CL-L1 in particular has been shown to activate complement (22). Elevated circulating CL-L1 concentrations have been observed in the early phase of acute liver failure and in cirrhosis (23). Also, evaluation of CL-L1 as a screening marker for colorectal cancer showed that higher levels of CL-L1 were associated with lower odds of colorectal cancer (24).

As the collectins have been shown to bind to different microorganisms and altered/exposed structures in the body (25), this new group of collections may also play a role in inflammatory conditions. In this study, we quantified serum concentrations of CL-L1 in children admitted to the pediatric intensive care unit (PICU), in comparison with those of healthy children, and explored the potential association with baseline characteristics and clinical outcomes of the critically ill children. We focused on the risk of acquiring a new infection and prolonged need for intensive care in view of the association we previously observed for low MASP-3 levels with these outcomes. We thus hypothesized that low levels of CL-L1 upon PICU admission, possibly induced by surgical, traumatic or other stress, are associated with a higher risk of acquiring a new infection and with a prolonged need for intensive care.

Results

Serum concentrations of CL-L1 in critically ill children and in healthy children

In propensity-matched cohorts of 81 critically ill and 81 healthy children, serum concentrations of CL-L1 were significantly lower in the critically ill children as compared with the healthy children (452 (372-541) ng/ml versus 561 (518-601) ng/ml, (median (IQR)), $p<0.0001$). The CL-L1 concentration in the propensity score-matched critically ill children did not differ from that in the non-matched children (466 (379-564) ng/ml, p=0.51). In the total group of critically ill children, the median CL-L1 serum concentration upon PICU admission was 466 (378-562) ng/ml. The CL-L1 concentration increased gradually during ICU stay to levels comparable with those in the healthy children (Figure 1).

CL-L1 serum concentrations upon PICU admission in relation to baseline characteristics

CL-L1 serum concentrations did not differ according to age, BMI, or medical history of diabetes or malignancy (data not shown). Cardiac surgery patients presented with higher baseline CL-L1 levels than patients admitted to the PICU for any other reason (469 (386-567) ng/ml in cardiac patients versus 441 (351-528) ng/ml in non-cardiac patients, $p=0.02$). CL-L1 concentrations upon PICU admission weakly correlated inversely with severity of illness, as evaluated by modified PRISM score (rho=-0.200, $p<0.0001$), PELOD score (rho=-0.106, $p=0.006$), or baseline concentrations of glucose (rho=-0.137, $p=0.0004$), lactate (rho=-0.257, $p<0.0001$), troponin (rho=-0.152, $p=0.004$) or Heart-type Fatty Acid Binding Protein (HFABP, rho=-0.317, $p<0.0001$). CL-L1 concentrations correlated directly with the concentrations of the other lectin pathway proteins (MBL, MASP-1, MASP-2, MASP-3, MAp44, M- and H-ficolin (17)) (Table 1), and inversely with CRP (rho=-0.136, $p=0.0006$). The correlation was particularly strong for MASP-1, MASP-3 and MAp44, which are three alternative splice products encoded by the same gene, *MASP1*.

Association of CL-L1 serum concentrations upon PICU admission with outcome of critical illness

In a multivariable logistic regression analysis, correcting for baseline characteristics, risk factors and the other lectin pathway proteins, a higher CL-L1 concentration upon PICU admission was independently associated with an increased risk of acquiring a new infection (OR 1.241 (1.031-1.494 95%CI), *p*= 0.02) (Table 2A). A low MASP-3 concentration upon PICU admission was independently associated with a higher risk of infection in this model (OR 0.701 (0.562-0.864 95%CI), *p*=0.001). This confirmed the association we previously observed for MASP-3, with an even lower odds ratio than in the model without CL-L1 (OR 0.809 (0.675-0.959 95%CI), *p*=0.02) (17). The independent associations of CL-L1 and MASP-3 with infection were maintained with the addition of the interaction between both protein concentrations to the model, with a *p*-value of 0.06 for the interaction variable (Table 2B).

In multivariable Cox proportional hazard analysis, correcting for baseline characteristics, risk factors and the other lectin pathway proteins, a high CL-L1 concentration tended to be associated with a prolonged time to PICU discharge (*p*=0.05, Table 3A). The hazard ratio of 0.933 (0.867-1.002 95% CI) indicated a lower likelihood of earlier alive discharge at any time with a higher CL-L1 concentration upon PICU admission. As for risk of infection, this association was in the opposite direction as that for MASP-3, showing a higher likelihood of earlier alive discharge at any time with a higher MASP-3 concentration (hazard ratio of 1.125 (1.046-1.205 95% CI, *p*=0.001). Again, this association was stronger than for the model without CL-L1 that was previously reported (HR 1.088 (1.020-1.157 95%CI), *p*=0.01) (17). Addition of the interaction between both proteins had no major effect, with a *p*-value of 0.92 for the interaction variable (Table 3B).

Discussion

In a large cohort of critically ill children, serum concentrations of CL-L1 upon PICU admission appeared to be lower than in age-, gender- and BMI-matched healthy controls. Corrected for baseline characteristics, risk factors and the other lectin pathway proteins, a higher CL-L1 concentration was associated with a higher risk of acquiring a new infection and with risk of a prolonged need for intensive care. The previously observed association of a low MASP-3 with a higher risk of these adverse outcomes was robustly maintained and became even stronger in this model with CL-L1.

To date, there is only very limited knowledge on the biological function of the pattern recognition molecule CL-L1. CL-L1 is encoded by the *COLEC10* gene and is primarily expressed in the liver, placenta and adrenal glands (18). Initially, CL-L1 was identified as a cytosolic protein but has now been found in the circulation, where it complexes with MBL-associated serine proteases (MASPs), the enzymes responsible for activation of complement through the lectin pathway (21, 22, 27). In healthy individuals, CL-L1 levels appear to be present at near adult levels at birth and show low variation (22). The few occurring fluctuations may have reflected periods of microbial challenge. The authors suggested that CL-L1 may have an important role during the first year of life, when other elements of the immune system are immature. After major surgery, a modest down-regulation of CL-L1 was observed that coincided with the CRP response (22). This is in agreement with the acute decrease in serum concentrations of CL-L1 that we observed in response to the severe stress of critical illness in children and correlated, although weakly, with the rise in CRP. The drop in CL-L1 in the critically ill children was more pronounced with increasing severity of illness.

We performed multivariable analyses to assess whether the upon PICU admission serum concentration of CL-L1 is associated with risk of infection and prolonged need of intensive care. We corrected for the classical baseline characteristics and risk factors. However, the innate immune system is an extremely complex system of intertwined pathways, whose adequate function depends on a very subtle balance. An extraordinary redundancy of most of the system components may mask a deficiency or

malfunctioning of an individual component. Moreover, several components display context specific properties, where they can act as dual function surveillance molecules, suppressing or enhancing inflammation, as e.g. shown for the lung collectins (26, 28). Therefore, we also corrected for the other lectin pathway proteins for which the concentrations upon PICU admission had been measured (17). These analyses revealed independent associations with infection and ICU stay for both CL-L1 and MASP-3. Intriguingly, these associations were in the opposite direction, despite both proteins being reduced in concentration upon PICU admission and a relatively strong correlation between both proteins. For CL-L1 a high concentration was associated with new infection and prolonged PICU stay, whereas for MASP-3 a low concentration was predictive for these adverse outcomes. This suggests that in the context of a sufficiently high MASP-3 concentration, having a high concentration of CL-L1 would be detrimental as it counteracts the protective effect of having a high MASP-3. This opposition by CL-L1 is further supported by the lower odds ratio for risk of infection and higher hazard ratio for time to alive discharge from PICU for MASP-3 when CL-L1 is entered in the model. Our analyses were not confounded by correlation between the variables in the model, as multicollinearity was carefully excluded. The two-sided nature of circulating CL-L1 in critical illness, being reduced as compared with healthy controls but higher concentrations being associated with infection and prolonged PICU stay, is intriguing. Unfortunately, lack of knowledge about the biological function of CL-L1 hampers speculation about the mechanisms that are involved. Clearly, basic scientific research will be needed to unravel the mechanistic processes that may underlie our observations. Also the immediate clinical relevance of the present findings is currently unclear. However, the data nicely illustrate the complex dualism of the inflammatory cascade, where apparently opposite forces are actually complementary and regulated in a subtle balance. This insight may contribute to a future of personalized medicine, where (a panel of) biomarkers may guide therapy towards enhancing inflammation (in case of infection) or suppressing inflammation (when excessive inflammation threatens organ function).

A major strength of the present findings is that we studied a large, heterogeneous population of (critically ill) children, which is quite unique. Indeed, most often clinical studies in ICU exclude children,

as they represent only a small population spread over different developmental stages, and results obtained from adult studies are too often extrapolated to children. Nevertheless, a limitation of our study is that we only showed associations, with no evidence of causality. Secondly, the measurements we performed are primarily of hetero-oligomers between CL-L1 and CL-K1 polypeptide chains, two collectins that have most likely evolved from the same ancestral gene by gene duplication (21, 27, 29). We have observed that there is a tight correlation between the amount of CL-L1 and CL-K1 in plasma but no one has studied in detail whether both molecules can be present separately at different sites in the body and whether such proteins appear in plasma at extreme conditions (24, 27, 29, 30).

In conclusion, corrected for baseline characteristics, risk factors and several lectin pathway proteins, a high serum CL-L1 concentration upon PICU admission is associated with an increased risk of infection and prolonged need of intensive care, and counteracts the protective effect on these outcomes of having a high MASP-3 concentration. More pathophysiological research will, however, be needed to further elucidate the role of CL-L1 during critical illness and during infection in particular. Nevertheless, the present findings yield valuable and unique information, which can incite further exploration in this relatively new, though expanding field of research.

Materials and methods

Subjects

This study is a pre-planned analysis of all patients (*n*=700) included in a randomized controlled study on tight glycemic control in the PICU (1). The majority of the children were admitted to the PICU after cardiac surgery for congenital heart defects (75%), whereas the others were admitted after complicated surgery or trauma, after solid organ transplant surgery, or after neurological, infectious or other medical disorders. Critically ill children were allocated upon PICU admission to either conventional insulin therapy (n=351, insulin was started only when the blood glucose concentration exceeded 215 mg/dl and was tapered down or stopped when glycemia fell below 180 mg/dl), or intensive insulin therapy (IIT, n=349, insulin was infused to achieve age-adjusted normal fasting glucose levels). Both randomization groups were comparable for baseline characteristics (1). The characteristics of the total patient cohort are shown in Table 4. In addition, we included 81 healthy children (Table 4), who were scheduled for minor ambulatory surgery. Blood was withdrawn preoperatively after catheterization that was required for induction and/or maintenance of anesthesia. These children were propensity score-matched with critically ill children (vide infra) to study the impact of critical illness. The study was approved by the KU Leuven Institutional Review Board (ML2586) and was registered with ClinicalTrials.gov (NCT00214916). The study was performed in accordance with the 1964 Declaration of Helsinki and its amendments. Written informed consent was obtained from the parents or legal guardians of patients and controls.

Blood sampling and biochemical analyses

Upon PICU admission, serum was collected and stored at -80°C until analysis. For 29 patients no baseline serum sample was available. For 40 patients, 20 per randomization group (with comparable baseline characteristics, data not shown), additional serum samples collected on day 2, day 4, day 8 (if

the patient was still in ICU on those days) and last day in PICU were used to study the time profile of CL-L1 during critical illness.

C-reactive-protein (CRP) was determined by routine clinical chemistry. Whole arterial blood glucose was measured at 1-4 hour intervals during ICU stay using the ABL700 blood gas analyzer (Radiometer Medical A/S, Copenhagen, Denmark). CL-L1 concentrations were measured in duplicate using an in-house time-resolved immune-fluorometric assay (TRIFMA) with a setup similar to conventional sandwich immune assays (22). However, we used a new and better characterized preparation of recombinant CL-L1, resulting in healthy control levels approximately three times lower than given in our original report (22). In brief, capture of the protein with anti-CL-L1 and detection of bound CL-L1 with biotinylated anti-CL-L1, was followed by incubation with Eu^{3+}-labeled streptavidin. After washing, the europium bound in the wells was quantified after addition of enhancement solution to the wells, and the fluorescence was read using time-resolved fluorometry. Data were analyzed using the WorkOut 2 software (PerkinElmer Life Sciences). In addition to a standard curve, consisting of serial dilutions of plasma, three internal control plasma samples (representing a high, medium and low concentration of the protein) and a buffer control were included on each microtiter plate. All samples were analyzed in duplicate and the analysis was repeated if the coefficient of variation was higher than 20%. The plate was repeated if the quality controls varied with a CV above 15% as compared with a collection of previously measured values. If the concentration of the samples exceeded the highest value of the standard curve, the assays were repeated at higher dilution.

Statistical analyses

Data were analyzed with JMP Pro 11.2.0 (SAS Institute Inc., Cary, NC). Two-sided *P*-values below 0.05 were considered significant. Data are presented as mean (95% CI), median (IQR), or proportion (percentage), as appropriate. Continuous variables were analyzed with the Wilcoxon test, and proportions with the chi-square test. Associations between variables were calculated with Spearman's correlation coefficient. To evaluate the association of CL-L1 serum concentrations upon PICU

admission with risk of infection, independent of known risk factors, multivariable logistic regression analysis was performed. The classical baseline characteristics and risk factors (age, gender, BMI, randomization, CRP, history of malignancy, cardiac surgery, modified pediatric risk of mortality (PRISM) score) were considered and in view of the potential context-specificity of disturbances in this system (26, 28) also the upon-admission concentrations of the other lectin pathway proteins (measured in (17)) were added. In a second step, the interaction between CL-L1 and MASP-3 concentrations was tested, in which the addition of the CL-L1 * MASP-3 interaction variable to the model assesses whether the effect of CL-L1 on risk of infection is different at different values of MASP-3. To evaluate the predictive power for likelihood to early alive discharge from PICU, Cox proportional hazard analysis was performed entering the same risk factors, with non-survivors censored beyond the longest staying survivor, ensuring the most stringent analysis avoiding competing risk between death and earlier discharge (31). We excluded multicollinearity of the variables entered in the models via the collinearity diagnostics tool in SPSS version 22.0 (32).

We used propensity-score matching of the SPSS R-menu (Version R2.10.1, R Foundation for Statistical Computing) and IBM SPSS Statistics 19 (SPSS Inc, Chicago, IL) to select demographically comparable cohorts of healthy and critically ill children (33, 34). Propensity-scores were estimated via logistic regression with age, gender and BMI as continuous covariates. One-to-one nearest neighbor matching was performed with use of a caliper of 0.004.

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Figure legend

Figure 1 : CL-L1 serum concentrations during critical illness

Time profiles of serum concentrations of CL-L1 are shown for 40 critically ill children, with CL-L1 concentrations measured upon PICU admission (adm), day 2 (D2), day 4 (D4), day 8 (D8) and last day in PICU. Data are expressed as box plots, where the central lines indicate the medians, the boxes the interquartile ranges and the whiskers the 10^{th} and 90^{th} percentiles.

Table 1: Correlation between concentrations of CL-L1 and other lectin pathway proteins upon PICU admission

	rho	***p***
MBL	0.095	0.01
MASP-1	0.461	<0.0001
MASP-2	0.175	<0.0001
MASP-3	0.564	<0.0001
MAp-44	0.528	<0.0001
H-ficolin	0.224	<0.0001
M-ficolin	0.214	<0.0001

Table 2: Multivariable logistic regression analysis for risk of new infection

	Corrected for baseline characteristics, risk factors and lectin pathway protein concentrations		Corrected for baseline characteristics, risk factors and lectin pathway protein concentrations and interaction	
	Odds ratio (95% CI) [a]	***p***	**Odds ratio (95% CI)** [a]	***p***
Age (per year added)	0.903 (0.853-0.954)	0.0004	0.907 (0.856-0.958)	0.0007
Gender (male)	1.088 (0.732-1.618)	0.67	1.120 (0.752-1.670)	0.57
BMI (per kg/m^2 added)	0.992 (0.954-1.028)	0.62	0.994 (0.955-1.030)	0.69
Randomized intervention (IIT)	0.658 (0.446-0.967)	0.03	0.646 (0.437-0.951)	0.02
Cardiac surgery	0.240 (0.133-0.427)	<0.0001	0.263 (0.145-0.473)	<0.0001
Malignancy	0.210 (0.065-0.606)	0.005	0.209 (0.064-0.606)	0.005
Modified PRISM-score (per unit added)	1.159 (1.114-1.208)	<0.0001	1.154 (1.109-1.203)	<0.0001
CRP (per mg/l added)	1.009 (1.004-1.015)	0.0008	1.009 (1.004-1.015)	0.001
MBL (per µg/ml added)	1.134 (0.950-1.355)	0.16	1.152 (0.964-1.377)	0.11
MASP-1 (per µg/ml added)	0.951 (0.881-1.025)	0.19	0.957 (0.886-1.031)	0.25
MASP-2 (per µg/ml added)	0.959 (0.174-5.246)	0.96	1.017 (0.183-5.670)	0.98
MASP-3 (per µg/ml added)	0.701 (0.562-0.864)	0.001	0.664 (0.530-0.824)	0.0003
MAp-44 (per µg/ml added)	1.163 (0.878-1.552)	0.29	1.177 (0.890-1.567)	0.25
M-ficolin (per µg/ml added)	1.073 (0.961-1.343)	0.38	1.086 (0.965-1.377)	0.37
H-ficolin (per µg/ml added)	1.019 (0.987-1.053)	0.26	1.022(0.990-1.056)	0.19
CL-L1 (per 0.1 µg/ml added)	***1.241 (1.031-1.494)***	***0.02***	***1.223 (1.015-1.475)***	***0.03***
CL-L1 * MASP-3 interaction				***0.06***

CI, confidence interval; CL-L1, collectin-L1; CRP, C-reactive protein; IIT, intensive insulin therapy; MAp, MBL-associated protein; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; PRISM, pediatric risk of mortality. [a] An odds ratio higher than 1 indicates an increased risk of infection. Hence, patients with a higher level of CL-L1 upon PICU admission have a higher risk of acquiring a new infection in PICU.

Table 3: Cox proportional hazard analysis for likelihood of early alive discharge from PICU.

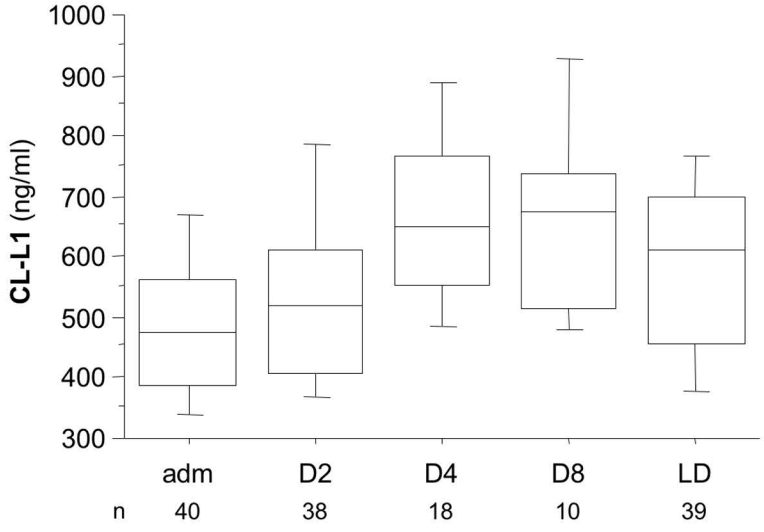
	Corrected for baseline characteristics, risk factors and lectin pathway protein concentrations		Corrected for baseline characteristics, risk factors and lectin pathway protein concentrations and interaction	
	Odds ratio (95% CI) [a]	***p***	**Odds ratio (95% CI)** [a]	***p***
Age (per year added)	1.039 (1.017-1.062)	0.0006	1.039 (1.017-1.062)	0.0007
Gender (male)	0.955 (0.811-1.124)	0.57	0.956 (0.811-1.127)	0.58
BMI (per kg/m^2 added)	0.997 (0.971-1.017)	0.79	0.997 (0.971-1.017)	0.79
Randomized intervention (IIT)	1.200 (1.020-1.411)	0.02	1.199(1.020-1.411)	0.02
Cardiac surgery	1.891 (1.471-2.451)	<0.0001	1.897 (1.465-2.477)	<0.0001
Malignancy	0.981 (0.627-1.479)	0.93	0.983 (0.628-1.484)	0.93
Modified PRISM-score (per unit added)	0.915 (0.898-0.931)	<0.0001	0.915 (0.898-0.931)	<0.0001
CRP (per mg/l added)	0.999 (0.997-1.001)	0.32	0.999 (0.997-1.001)	0.32
MBL (per µg/ml added)	1.040 (0.961-1.121)	0.32	1.040 (0.961-1.123)	0.32
MASP-1 (per µg/ml added)	1.005 (0.974-1.036)	0.77	1.005 (0.974-1.036)	0.76
MASP-2 (per µg/ml added)	0.989 (0.497-1.883)	0.97	0.988 (0.497-1.882)	0.97
MASP-3 (per µg/ml added)	1.125 (1.046-1.205)	0.001	1.123 (1.040-1.211)	0.003
MAp-44 (per µg/ml added)	0.968 (0.847-1.097)	0.61	0.968 (0.847-1.098)	0.62
M-ficolin (per µg/ml added)	1.013 (0.957-1.051)	0.59	1.013 (0.957-1.051)	0.58
H-ficolin (per µg/ml added)	0.998 (0.986-1.010)	0.77	0.998 (0.986-1.010)	0.79
CL-L1 (per 0.1 µg/ml added)	***0.933 (0.867-1.002)***	***0.05***	***0.932 (0.865-1.003)***	***0.06***
CL-L1 * MASP-3 interaction				***0.92***

CI, confidence interval; CL-L1, collectin-L1; CRP, C-reactive protein; PICU, pediatric intensive care unit; IIT, intensive insulin therapy; MAp, MBL-associated protein; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; PRISM, pediatric risk of mortality. [a] A hazard ratio above 1 indicates a higher likelihood of earlier discharge alive from the ICU at any time. Hence, higher upon-admission CL-L1 levels increase the risk of an extended stay in the PICU.

Table 4: Patient characteristics

	Total patient population	**Propensity score matched population**	
	n=700	**Critically ill children** *n*=81	**Healthy children** *n*=81
Baseline characteristics			
Demography			
Gender (male) (*n*, %)	401 (57.3)	53 (65.4)	46 (56.8)
Age (years) (median - IQR)	1.3 (0.3-5.0)	3.3 (1.6-4.7)	3.6 (1.6-5.3)
BMI (kg/m^2) (mean - 95%CI)	15.4 (15.0-15.8)	15.8 (15.3-16.3)	16.2 (15.7-16.6)
Medical history			
Malignancy (*n*, %)	36 (5.1)	7 (8.6)	
History of diabetes mellitus (*n*, %)	6 (0.9)	1 (1.2)	
Diagnostic category			
Cardiac surgery for congenital heart defects (*n*, %)	526 (75.1)	56 (69.1)	
Complicated surgery or trauma (*n*, %)	74 (10.6)	10 (12.3)	
Neurological medical disorders (*n*, %)	21 (3.0)	4 (4.9)	
Infectious medical diseases (*n*, %)	32 (4.6)	5 (6.2)	
Other medical disorders (*n*, %)	34 (4.9)	4 (4.9)	
Solid organ transplants (*n*, %)	13 (1.9)	2 (2.5)	
PELOD first 24h (median - IQR)	11 (2-12)	11 (2-12)	
PRISM [a] (median – IQR)	10 (7-14)	8 (6-13)	
CRP admission (mg/l) (median - IQR)	28.3 (15.2-77.8)	38 (22-56)	
Admission blood glucose (mg/dl [b]) (median - IQR)	129 (98-176)	132 (112-161)	
Randomisation to intensive insulin therapy, (*n*, %)	349 (49.9)	48 (59.3)	
Outcome parameters			
PICU mortality (*n*, %)	29 (4.1)	3 (3.7)	
Days in PICU (median - IQR)	3 (2-7)	2 (2-4)	
Secondary infection (*n*, %)	231 (33)	19 (23.5)	
Peak bilirubin >1.5 mg/dl [c] (*n*, %)	140 (20.0)	10 (12.3)	
Days on mechanical ventilation (median - IQR)	2 (1-5)	1 (1-2)	
Days on vasoactive drug support (median - IQR)	2 (0-4)	1 (0-2)	

CI, confidence interval; CRP, C-reactive protein; PICU, pediatric intensive care unit; IQR, interquartile range; PELOD, Pediatric Logistic Organ Dysfunction; PRISM, Pediatric Risk of Mortality. [a] Modified Pediatric Risk of Mortality score: PRISM score calculated with only the admission value of blood glucose, which is unaffected by the study intervention. [b] To convert glucose to mmol/l, multiply by 0.05551. [c] To convert bilirubin to µmol/l, multiply by 17.1.


CL-L1 (ng/ml)
1000
900
800
700
600
500
400
300
adm
D2
D4
D8
LD
n
40
38
18
10
39